

Standard Operating Procedure

Laboratory Procedures for Chlorophyll *a*

Analysis in Water






Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

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SCOPE AND APPLICABILITY

This document outlines procedures for the analysis of water column chlorophyll *a* samples that are collected by Kentucky Division of Water field personnel engaged in ambient water quality monitoring in lakes, streams, rivers and wetlands. Chlorophyll *a* concentration is used as a surrogate for sestonic algal biomass and is a factor in calculating lake trophic status index (TSI). Although samples collected from surfaces (i.e., periphyton) can be analyzed with similar methods, this SOP is specifically oriented toward water column samples. A Turner Trilogy fluorometer is used in sample analysis. Use of other instruments may require different procedures than those described here.

SUMMARY

This method follows those described in USEPA 445 r1.2 (USEPA 1992) and SM 10200H (APHA 1989). Samples are filtered within 24 hours of collection onto glass fiber filters and placed in a freezer for storage up to 24 days. Filters are extracted in 90% acetone, ground mechanically using a tissue grinder, and the slurry centrifuged to clarify. Extract is transferred to a cuvette and analyzed using a fluorometer with narrow bandpass filters (Turner Designs Trilogy digital fluorometer with Chl NA optical kit). This method results in a measurement of the concentration of chlorophyll-*a* with minimal interference from phaeophytin *a* and other chlorophylls and with no acidification step (i.e. the “modified fluorometry method” described in USEPA 445 rev 1.2, otherwise known as the Welshmeyer method).

ACRONYMS

CCV: Continuing Calibration Verification
EDL: Estimated Detection Limit
FD: Field Duplicate Sample
DIW: De-ionized Water
HAZCOM: Hazard Communication
KDEP: Kentucky Department for Environmental Protection
KDOW: Kentucky Division of Water
LRB Laboratory Reagent Blank
LSS Laboratory Split Sample
RFU: Relative Fluorescence Units
QA: Quality Assurance
QC: Quality Control
SOP: Standard Operating Procedure

HUMAN HEALTH AND SAFETY

- The grinding of filters during the extraction step must be conducted in a fume hood due to the volatilization of acetone by the tissue grinder. Ensure that the hood sash is set to the inspection mark.
- Store all containers with acetone in the flammables cabinet when not in use.
- Gloves, lab coat, and eye protection should be worn during grinding of filters or during other steps that present a risk of splashing of acetone.
- A heavy glove should be worn when grinding samples to protect the hand in case of grinding tube failure. Eye protection also should be worn.
- Disposal of glass consumables should be done with care, using a sharps container where appropriate.
- If using dry ice to store filters in the field, handle with gloves and keep dry ice in paper wrapping. Use a cooler with a lid that is not airtight to allow gases to escape. Keep cooler in well ventilated area at all times, such as in the vehicle rooftop carrier or on boat. Do not keep inside vehicle or indoors where people are present for more than short periods of time.

PERSONNEL QUALIFICATIONS / RESPONSIBILITIES

Personnel performing this method will be trained in this SOP by experienced staff. Training will consist of verbal and in-person instructions including demonstration of safe work practices. Personnel must complete the KYDEP HAZCOM training as required by DEP before performing this method. Personnel also should have fully read and understood the user manual for the Turner Trilogy fluorometer and the IEC HN-SII centrifuge.

Personnel performing this method for the first time will demonstrate proficiency in the extraction of sampled filters prior to analyzing field samples for projects. A 1L water sample will be obtained from a natural waterbody that is expected to have a chlorophyll concentration at least 10X the IDL. Ten replicate filters will be extracted and analyzed following the procedures in this SOP. The percent relative standard deviation of chlorophyll-*a* concentrations should not exceed 15%. Percent relative standard deviation is calculated as the standard deviation divided by the mean, multiplied by 100.

EQUIPMENT AND SUPPLIES

Table 1 contains a list of supplies required for Chlorophyll *a* laboratory analysis.

Table 1. Laboratory Equipment and Supplies

Sample Containers

250mL opaque (brown) bottles, or larger as needed

Laboratory Equipment

Turner Trilogy fluorometer with Chl NA lamp installed

Turner Adjustable Secondary Standard, Turner 8000-952

6 position centrifuge, capable of 675 g

Vacuum pump with gauge capable of maintaining a vacuum up to 6 in Hg

Filtration apparatus, glass or acetone-resistant plastic, with 47-mm disk base

Tissue grinder assembly

Teflon serrated pestles with matching 50 ml borosilicate mortars (Thomas Size C or similar)

Drive motor on laboratory stand

Graduated cylinders: 100 ml, 500 ml, 1000 ml

Volumetric pipets: 1mL, 10 mL

Filter forceps

Scissors

Test tube racks to fit centrifuge tubes and cuvettes

Polyethylene squirt bottles

Low wattage lamp

Fume hood

Refrigerator

Freezer

Laboratory Supplies

Chlorophyll calibration standard, Turner 10-850

15mL graduated centrifuge tubes with screw caps

Plastic transfer pipets

12mm x 75mm borosilicate cuvettes

Alconox® detergent

Acetone, HPLC grade

Powderless latex/nitrile gloves

glass microfiber filters, 47mm diameter, pore size 0.7 µm (Whatman GF/F or similar)

De-ionized water

Laboratory wipes (e.g., Kim wipes®)

Aluminum foil

Gallon zip lock bags

Sharps box

Additional supplies and Equipment for off-site filtration and storage

Vacuum hand pump and tubing

Gallon storage bags

Field freezer or dry ice and cooler

METHOD

Cautions

- Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of chlorophyll *a*.
- Quenching effects are observed in highly concentrated solutions. The linear range per manufacturer specifications is 0-300µg/L. Samples reading greater than 250 µg/L should be diluted and re-run. The need for dilution can be minimized by avoiding filtering too much volume of highly concentrated samples.
- Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, standards, and LRBs must be at the same temperature to prevent errors and/or low precision. Analyses of samples at ambient temperature is recommended in this method. Ambient temperature should not fluctuate more than $\pm 3^{\circ}\text{C}$ between calibrations or recalibration of the fluorometer will be necessary.
- All photosynthetic pigments are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20°C to prevent degradation.
- Gloves must be worn when handling samples, filters, and glassware; all glassware must be clean and acid-free to avoid degradation of chlorophyll by contact with acidic substances. Also, samples from acidic waters should be filtered and analyzed as soon as possible for this reason.
- The grinding pestles eventually become worn and cease to function correctly. Inspect pestles regularly and replace when signs of wear are apparent or when filter material becomes difficult to grind without producing noticeable heat. Signs of worn pestles are loose fit inside grinding tubes, visibly degraded grooves, and excessive wobble when spun by the motor.

Instrument Initial Calibration, Continuing Calibration Verification, and Determination of Estimated Detection Limit

The fluorometer is calibrated shortly before the beginning of the sampling season (Initial Calibration). Additionally, the fluorometer must be calibrated any time the optical kit is removed, or any time the continuing calibration verification (CCV) fails to meet acceptance criteria. Calibration standards are available from the Turner that have been quantified spectrophotometrically and can be used to perform a two-point calibration. The standards are shipped overnight and must be immediately placed in the freezer upon receipt. Per the

manufacturer, the standards are good for one year if unopened and 1 month once opened, provided they are properly stored in the dark and at -20°C. Once the calibration has been performed (see User Manual, Turner 2016), measure the fluorescence (as µg/L chlorophyll *a*) of the solid secondary standard following instructions in the instrument manual. The solid secondary standard is used for continuing calibration verification (CCV), which serves to detect possible drift in the instrument (readings should be +/-10% of established value). Checking the calibration with the secondary standard is done at the beginning and end of each batch. This check is also recommended prior to initiating sample extractions to allow time for the instrument to be recalibrated if necessary before proceeding. Record the calibration details in the fluorometer calibration and maintenance log located in the chlorophyll lab (Appendix C).

The Estimated Detection Limit (EDL) is determined annually. After calibration has been performed, at least four blank filters are processed similar to samples, and the measured relative fluorescence values averaged. A chlorophyll *a* solution of known concentration (usually the low-end calibration standard) is serially diluted until it yields a response (RFU) that is approximately 3x the average response of the blank filters. This concentration is the EDL. Record the details of EDL determination and the final value on the fluorometer calibration log.

*To perform serial dilutions, use 1, 5 and 10 mL volumetric pipets.

Other Equipment Maintenance and Operation

The vacuum pump requires periodic maintenance. See pump owner's manual for maintenance instructions and also how to reset maximum pressure if the setting is lost.

Sample Collection

- Water samples are sampled per project plan requirements. Use opaque sample containers or wrap with foil to protect from light.
- Store water samples on wet ice immediately after collection.
- Record sample details on a Chain of Custody (COC) form that contains all required elements listed in Appendix B.
- Samples should be filtered within 24 hr of collection. Samples from eutrophic waters with high levels of algae or samples from acidic waters should be filtered as soon as possible. If samples cannot be returned to the laboratory for filtering within 24 hr (i.e., during overnight sampling trips) then samples should be filtered off-site (e.g., in the hotel at the end of the work day or before starting the next day) and the filters placed in a portable freezer or on dry ice for storage until filters can be transferred to the laboratory freezer. Wet ice storage of filters is acceptable for up to 4 hours.
- If samples are filtered off-site include filtering details on the COC. This information will be transcribed to the sample log when samples are received in the laboratory.

Receive Field Sample into Laboratory

1. Inspect sample container for any damage.
2. Review sample Chain of Custody (COC) for completeness of all required information (Appendix B).
3. Assign laboratory ID to sample. The laboratory ID will be maintained throughout the analysis of the sample in the laboratory and will serve as a unique identifier to the sample. All samples, including any QC samples (i.e. laboratory duplicates, laboratory reagent blanks, etc.), will receive a laboratory ID. IDs will be formatted as “C”, the year (YY) and a sequential number. For example the first laboratory ID for calendar year 2017 will be: C17-001. (See later sections for ID conventions for QC samples)
4. Log Sample in to Chlorophyll a Sample Log (Appendix C). Transcribe filtering information to log in the case of samples filtered off site and delivered to the lab as filters. Keep the log continuous and mark out unused rows if skipping rows to start a new sheet.

Preparation of Aqueous Acetone Solution -- 90% acetone /10% water

1. Measure 100 mL of water with a 100 mL graduated cylinder.
2. Transfer to a 1-L flask or storage bottle.
3. Measure 900 mL of acetone into a 1000 mL graduated cylinder
4. Transfer acetone to the flask or bottle containing the water.
5. Mix, label and store.

Sample Filtration, Grinding and Extraction, and Steeping

Sample Filtration

1. Assemble the filtration apparatus and use clean forceps to place a filter onto the base. Attach the vacuum source with vacuum gauge and regulator.
2. Adjust workspace lighting to the minimum that is necessary to read instructions and operate instrumentation. Keep samples in the refrigerator/cooler until ready to filter.
3. Prior to drawing a subsample from the container, thoroughly but gently agitate the container to suspend the particulates (invert several times).
4. Quickly pour a portion of sample into a graduated cylinder and accurately measure the volume. Volumes for Kentucky’s waters that typically produce a mid-range chlorophyll-*a* value are 50-100 mL. Larger volumes may be required in oligotrophic waters. Do not filter less than 50 mL. **Record sample volume on chlorophyll a sample log (see Appendix B).**
5. Pour the subsample into the filter tower of the filtration apparatus and apply a vacuum. Vacuum filtration should not exceed 6 in. Hg (20 kPa). Higher filtration pressures and excessively long filtration times (> 10 min) may damage cells and result in loss of chlorophyll.
6. A sufficient volume has been filtered when a faint but visible green or brown color is apparent on the filter.

7. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.
8. Remove the filter from the base with tweezers, fold once with the particulate matter inside and lightly blot the filter with a tissue if necessary to remove excess moisture. Place filter in 15 mL centrifuge tube. Using a grease pencil, mark centrifuge tube with the laboratory ID. Note: acetone used in the grinding process will remove ink from the tubes so Sharpies® should not be used. Transfer to freezer, using plastic storage bags to keep sets of samples together and label with sample date.
9. If filtering numerous samples, the tubes can be placed on wet ice until the batch is complete (or up to 2-4 hr), but filters should be stored at -20°C as soon as possible.
10. If filtering off-site, folded filters can instead be placed in foil, folding edges over to protect. Label foil pack or centrifuge tube with the station and date, place in plastic storage bag, and place in portable freezer or on dry ice. Foil packs are preferred because they can be wedged between freezer packs to facilitate more effective freezing during periods when the portable freezer is not running (i.e., when parked for extended periods).
11. Clean filter apparatus by washing in laboratory grade detergent and water, rinsing well with tap water, and finally triple-rinsing with deionized water. Blot dry with a laboratory tissue and repeat for additional sites.
12. At least 1 in 20 samples must be duplicated by filtering a second aliquot of sample. This is the Laboratory Split Sample (LSS). Assign the sample ID similar to the regular/original but add the suffix "LSS". Plan ahead to ensure that at least one sample per batch will have sufficient volume to allow splitting (e.g. use larger sample container if necessary).
13. Preservation -- filters should be stored frozen (-20°C) in the dark until extraction.
14. Holding Time -- Filters can be stored frozen at -20°C for as long as 3½ weeks (24 days) without significant loss of chlorophyll a.

Grinding and Extraction

1. Note: It is recommended to check the calibration of the fluorometer using the solid secondary standard prior to initiating sample extractions so that issues can be addressed or the instrument can be recalibrated before proceeding. The reading should be +/- 10% of the original value.
2. If filters have been frozen, remove them from the freezer when ready to process (no more than a few at a time) but keep in the dark and/or wrapped.
3. Set up the tissue grinder assembly in the hood and have on hand laboratory wipes and squirt bottles containing DI water and 90% acetone.
4. Adjust workspace lighting to the minimum that is necessary to read instructions and operate instrumentation.
5. Remove a filter from its container, cut into small pieces with scissors and place in the glass grinding tube. Push into the bottom with a clean probe or glass rod.
6. Using a squirt bottle, add ~6 mL 90% acetone solution to the grinding tube.

7. Seat the grinding tube onto the pestle submerging the pestle in the acetone. Turn on the motor and gently move the tube up and down to repeatedly bring the pestle end to the bottom and force the filter fragments to the side walls to be ground. (NOTE: Light to moderate pressure should be sufficient and there should not be noticeable heat build-up. The grinding should take 30 seconds or less. If more time or pressure seems necessary then inspect pestle for signs of wear – see Cautions section.)
8. Pour the slurry into a labeled 15-mL screw-cap centrifuge tube. Using a fine-tipped squirt bottle of 90% acetone, rinse the pestle into the grinding tube and rinse the grinding tube walls, transferring the rinsate into the centrifuge tube. Repeat as necessary, taking care to use as little acetone as possible as not to exceed the 15 mL capacity of the centrifuge tube.
9. Using the squirt bottle of acetone, bring the total volume up to 15mL by reading graduations on the centrifuge tube.
10. **Record the final extract volume in chlorophyll a sample log (usually 15 mL).**
11. Cap the tube tightly and shake it vigorously. Place foil-covered tube in test tube rack and place rack in refrigerator (4°C).
12. Thoroughly clean the pestle, grinding tube, and probe/rod by triple-rinsing with deionized water and rinsing a final time with acetone. Carefully inspect the pestle and rod for any residue that may be adhering and remove with a clean tissue. Clean the forceps and scissors.
13. Proceed to the next filter and repeat the steps 5-12 above. If batch is more than 20 samples then do next step (LRB) after every 20th sample before proceeding.
14. After all regular samples have been processed (or after every 20th sample in the processing batch if batch is more than 20) prepare a laboratory reagent blank (LRB) by repeating steps 5-12 using a clean blank filter. Assign this sample an ID in the form CYY-LRB-MMDDYY (e.g., C17-LRB-040117), where the date is the date that the LRB is prepared. If there is more than one LRB in a day then add a sequential number to the end of the ID.

Steeping

1. Samples should be allowed to steep for a minimum of 2h but not to exceed 24h.
2. The tubes should be shaken at least once during the steeping period.
3. After steeping, proceed to Fluorometric Analysis.

Fluorometric Analysis of Chlorophyll a Samples

1. Thirty minutes to one hour prior to analyzing in the fluorometer, remove rack of steeped samples from the refrigerator and set in a dark place to allow samples to warm to room temperature.
2. Adjust workspace lighting to the minimum that is necessary to read instructions and operate instrumentation.
3. After samples have come to room temperature, load tubes into the centrifuge and spin for 5 minutes @ 1000 g (2250 rpm with the IEC HN-SII centrifuge). Note: always

balance the centrifuge by filling all 6 holders, installing “dummy” centrifuge tubes if necessary that are filled to the same volume as the samples).

4. Turn the fluorometer on and use the touch screen to register that the Chl-NA optical kit is in place.
5. Hit Calibrate and choose the last valid stored calibration.
6. Pipet 2-3 mL of 90% acetone into a cuvette, wipe with a Kim wipe® and place in the round receiver. Hit “measure fluorescence” and enter 1 mL for sample volume and extract volume. Verify that the measured value is 0.0.*
7. Remove the round cuvette adapter from the optical kit and insert the solid secondary standard, keeping the tab in front. Select “measure fluorescence” and enter 1 mL for sample volume and extract volume. **Record the measured value for the starting CCV on the chlorophyll sample log (QC Type = CCV-S).** Compare this to the value determined during the last calibration recorded in the calibration and maintenance log. The value should be +/- 10% of this value. If it is not, then locate source of problem and rectify before proceeding. Replace the round cuvette adapter when finished.
8. Remove a centrifuge tube from the centrifuge and place in the test tube rack, being careful not to disturb the pellet of filter residue.
9. Using a plastic transfer pipet, remove 2-3mL of sample from the centrifuge tube without disturbing the pellet and transfer to a clean cuvette.
10. Wipe the cuvette with a laboratory wipe and place in the receiver.
11. Select “measure fluorescence”. Enter sample volume and extract volume when prompted. The chlorophyll *a* concentration in µg/L will be displayed. **Record this value in the chlorophyll a sample log.** If “OVER” is displayed then sample is too concentrated and must be diluted. Judge the amount of dilution necessary and follow instructions in next section (Sample Dilutions). **If no dilution then record dilution factor as 1.**
12. Remove the cuvette from the fluorometer and pour sample into labeled acetone waste container located in the fume hood.
13. Repeat steps 9 through 13 as needed to complete all regular samples, the LSS (if applicable) and the LRB. If batch is more than 10 samples then do next step (CCV) after every 10th sample before proceeding.
14. When the batch is complete (or after every 10 samples), measure the CCV. Place the solid secondary standard in the receiver and enter volume and extract volume of 1. **Record the measured value of the ending CCV on the chlorophyll sample log (QC Type = CCV-E).** The value should be +/- 10% of the original value. If it is not then locate source of problem and rectify and re-run samples if possible. If samples cannot be re-analyzed and the issue cannot be resolved then samples must be flagged.
15. **For any flagged samples, record details about the nature of the flag on the sample log.** Use F and then a sequential number for flags and then explain in the space provided at the bottom.
16. When finished, turn off the fluorometer and discard all empty cuvettes and pipettes in the sharps box. Pour remaining sample left in centrifuge tubes into the acetone waste and discard tubes.

17. At the end of a batch of samples (or when waste acetone container is full) arrange with Environmental Services Branch staff for waste acetone to be taken to the central acetone waste storage.

*NOTE: If the fluorometer does not read 0.0, clean or replace the cuvette and read again. Prepare fresh acetone solution if necessary. If the fluorometer still does not read 0.0, flag the samples in the batch and record the blank value in the flag comments. If the issue cannot be resolved re-calibrate the fluorometer before analyzing additional samples procedures (Turner Designs 2016).

Sample Dilutions

Label a clean centrifuge tube with the sample ID and the dilution factor. Dilution factors are written as final volume / volume of sample (e.g. 5 mL of sample diluted to a total of 10 mL with 5 mL acetone has dilution factor of 2). Record the dilution factor on the sample log. Transfer the desired amount of sample using a 1, 5 or 10 mL pipet, from either the cuvette or the remainder of sample in the original centrifuge. Using a clean 1 or 10 mL pipet measure the desired amount of 90% acetone into the centrifuge tube. Mix thoroughly with a clean plastic transfer pipet before transferring to cuvette for measurement. Use these amounts as a general guideline for typical dilutions: x2 dilution use 5 mL sample and 5 mL 90% acetone; for x3 dilution use 3 mL sample and 6 mL 90% acetone; for x4 dilution use 3 mL sample and 9 mL 90% acetone.

DATA AND RECORDS MANAGEMENT

All sample information is logged into the chlorophyll a sample log (Appendix B). Final chlorophyll results are entered in KWADE as soon as possible after analysis and the log filed in the appropriate project folder. Calibration logs (Appendix C) are kept in the chlorophyll lab for review and are scanned for filing in project folders.

QUALITY CONTROL AND QUALITY ASSURANCE

- The linear dynamic range and instrument method detection limit of the Trilogy fluorometer have been determined by the manufacturer to be 0-300 µg/L and 0.025 µg/L.
- The estimated detection limit (EDL) is determined annually.
- Personnel will demonstrate proficiency in sample extraction when performing this method for the first time.
- The fluorometer will be calibrated with a chlorophyll *a* standard at the beginning of the season, if the optical kit is removed, or if the CCV fails to meet acceptance criteria.
- Continuing calibration verification (CCV) will be accomplished using the Turner solid secondary standard, measured at the start and end of each sample analysis batch (the sample extracts analyzed in a day), and additionally after every 10 samples for large batches.

- A laboratory reagent blank (LRB) will be analyzed by extracting, processing and analyzing a blank filter with each sample processing batch (the set of filters processed in a day), or additionally after every 20 samples for large batches.
- A laboratory split (a second filter processed from the same sample) will be performed at a rate of at least 1 in 20 samples filtered for a project.
- Field duplicate samples are generally recommended at a rate of 1 in 20 samples (5%) to assess repeatability of field collections. Field duplicates should be specified in program management plans or quality assurance project plans along with their acceptance criteria.
- QAQC requirements, acceptance criteria, and corrective actions are summarized in Table 2.

QAQC requirements for chlorophyll-*a* analysis

QC Requirement	Frequency	Acceptance Criteria	Corrective Action
Extraction Proficiency Demonstration	Each analyst, prior to analyzing samples for the first time	<15% Relative Standard Deviation (std dev / mean x 100) (10 split samples)	Retrain and repeat until pass
EDL determination	Annually	EDL must meet DQOs for monitoring programs	n/a
Initial Calibration	Annually, after optical kit removal, or after CCV fail	Successful calibration following fluorometer user manual instructions	If calibration is not successful then locate source of problem and rectify before proceeding with analyses.
Continuing Calibration Verification (CCV)	At beginning and end of each sample batch; plus every 10 samples for large batches.	CCV Recovery = 100 +/- 10	If outside acceptance criteria, locate source of problem and rectify. Recalibrate and re-analyze all samples if possible; if not possible then flag samples as biased high or biased low accordingly.

QC Requirement	Frequency	Acceptance Criteria	Corrective Action
Laboratory Reagent Blank (LRB)	One per batch; plus one in 20 samples for large batches.	Concentration < EDL	If concentration > EDL then investigate sources of contamination in the laboratory; if LRB values are $\geq 10\%$ of chlorophyll values in samples then samples must be reanalyzed after acceptable LRB is achieved or results must be flagged.
Laboratory Split Sample (LSS)	At least one per 20 samples per project.	RPD $\leq 20\%$ (if sample concentrations are >EDL)	If outside limits then investigate sources of variability; retraining and proficiency testing should be considered if LSS is repeatedly out of bounds for an analyst.

REFERENCES

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- Turner Designs. 2016. "Trilogy Laboratory Fluorometer User's Manual. Version 1.3, P/N 998-7210." Sunnyvale, CA. <http://www.turnerdesigns.com/t2/doc/manuals/998-7210.pdf>.
- US Environmental Protection Agency. 1992. Method 445.0 rev. 1.2: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater phytoplankton by fluorescence. Cincinnati, OH: Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency.

APPENDIX A: CHAIN-OF-CUSTODY REQUIREMENTS

Sample chains of custody may be formatted as appropriate to best meet project management needs. At minimum the Chain of Custody must contain the following information:

- A header that identifies the sample as a chlorophyll sample and lists the project or program name.
- KWADE Station ID (where applicable) or GPS coordinates if not an established station
- Sample Location Description
- Sample Primary Collector
- Collection Date and Time
- Sample Type (Field Sample, Field Duplicate, or other type)
- Collection Method (Vertical Discrete Composite, Grab-Direct to Sample Container, Grab-Using Sampler, or other method)
- Collection Depth or Depth Zone (e.g. Euphotic Zone)
- Container Type and Capacity
- Storage (will always be wet ice)
- Check box to identify if sample filtered prior to delivery to lab (off-site)
- Filtering details (where applicable)
 - Date/Time Filtered
 - Filtered By Person
 - Volume Filtered
 - Filter Storage (portable freezer, dry ice, wet ice)
- Blocks for date/time sample relinquished and relinquished by, date/time sample received and received by.
- Lab Sample ID (for lab use when samples logged in to Chlorophyll-a sample log)

APPENDIX B: EXAMPLE CHLOROPHYLL A LOG

Project/Year: _____											Page ____ of ____		
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	Page ____ of ____		
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APPENDIX C: FLUOROMETER CALIBRATION LOG

Fluorometer Calibration Log: Turner Trilogy, Division of Water Frankfort Lab

Calibration Date: _____ Performed By: _____

Standard Lot #: _____

Prepared Date: _____ Received Date: _____

Low standard concentration: _____ $\mu\text{g/L}$

High standard concentration: _____ $\mu\text{g/L}$

Calibration:

	RFU	Reading after
blank		
low		
high		

File Name: _____

Solid Secondary Standard fluorescence: _____ $\mu\text{g/L}$

CCV acceptance range: _____ - _____ $\mu\text{g/L}$

Filter Blanks RFU:

_____, _____, _____, _____, _____; average: _____ ; 3x average: _____

Low Standard Dilutions:

Dilution Factor	Calculated Concentration	RFU

Estimated Detection Limit: _____ $\mu\text{g/L}$